Supporting Information

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SI Methods

Antibodies Used in Immunoblotting or Immunoprecipitations (Indicated Dilutions Are for Immunoblotting). AR (1:1000, #06–680 Upstate); acetylated lysine (#05–515 Upstate); acetylated tubulin (1:2000, #T6793 Sigma); alpha-tubulin (1:10,000, #T6074 Sigma); beta-actin (1:5000, #A5441 Sigma); ERG-1/2/3, (1:5000, #sc-354X Santa Cruz); FLAG epitope (#F7425 Sigma); GAPDH (1:5000, #sc-32233 Santa Cruz); HDAC6 (1:1000, #sc-11420 Santa Cruz); HSP90 (1:1000, #SPA-840 Stressgen); mouse IgG (#sc-2025 Santa Cruz). The following secondary antibodies were used: anti-mouse IgG (1:5000, #A6782 Sigma); anti-rabbit IgG (1:3000, #NA934 Amersham or 1:10,000, #sc-2004 Santa Cruz).

ChIP PCR Primer Sequences and Conditions. *PSA* ARE ChIP Sense Primer: GCCTGGATCTGAGAGAGATATCATC

PSA ARE ChIP Anti-Sense Primer: ACACCTTTTTTTT-TCTGGATTGTTG

TMPRSS2 ARE ChIP Sense Primer: TGGTCCTGGAT-GATAAAAAAAGTTT

TMPRSS2 ARE ChIP Anti-Sense Primer: GACATACGC-CCCACAACAGA

Primers were synthesized by Integrated DNA Technology (IDT). ChIP PCR program consisted of: 95 °C for 5 minutes for 1 cycle; 95 °C for 30 seconds, 58 °C (*TMPRSS2*) or 60 °C (*PSA*) for 30 seconds, and 72 °C for 30 seconds for 35–40 cycles; 72 °C final extension for 5 min.

Real-time PCR Primer Sequences and Conditions. Taqman gene expression assays for *AR* (Hs00171172_m1), *PSA* (*KLK3*, Hs02576345_m1), *HDAC6* (Hs00195869_m1), and *18S* endogenous control (4319413E) were purchased from Applied Biosystems.

TMPRSS2-ERG QRTPCR Sense Primer: CGCGAGCTA-AGCAGGAG

TMPRSS2-ERG QRTPCR Anti-Sense Primer: CGACTG-GTCCTCACTCACAA

TMPRSS2-ERG QRTPCR Probe: FAM5′-CGCGGCAG-GAAGCCTTATCAG-3′TAMRA

Primers and probe for *TMPRSS2-ERG* fusion were purchased from IDT. The real-time PCR program consisted of: 50 °C for 2 min for 1 cycle; 95 °C for 10 min for 1 cycle; 95 °C for 15 seconds; and 60 °C for 15 seconds for a total of 40 cycles.

siRNA Sequences. *HDAC6* gene: CTGCAAGGGATGGATCT-GAAC

Nontargeted control *luciferase* gene: CGTACGCG-GAATACTTCGA

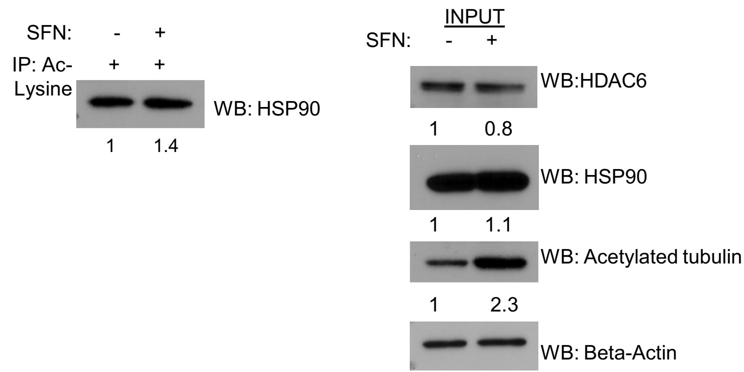

Fig. S1. Sulforaphane treatment of VCaP prostate cancer cells increases HSP90 acetylation in VCaP cells. An immunoprecipitation with an anti-acetyl lyine antibody was carried out after treatment with sulforaphane (SFN) 20 μM or vehicle for 6 h followed by a Western blot for HSP90. Enrichment was quantified for each immunoprecipitation. Inputs were probed with the indicated antibodies by Western blot and were quantified.

Fig. S2. CDDO-Imidazole recapitulates sulforaphane's effects. (*A*) LNCaP and (*B*) VCaP cells were treated with vehicle or increasing concentrations of CDDO-Imidazole for 24 h. Protein lysates were probed with the antibodies indicated. AR, acetylated tubulin, HDAC6, and actin levels were quantified by Western blot.

Fig. S3. High-dose sulforaphane treatment of prostate cancer cells lowers *AR* transcript levels. (*A*) Real-time PCR of *AR* gene expression with cDNA from LNCaP cells treated with vehicle, increasing doses of sulforaphane, or TSA. (*B*) Real-time PCR of *AR* gene expression with cDNA from VCaP cells treated with vehicle, increasing doses of sulforaphane, or TSA at 24 h. The vehicle-treated sample was set to 1. *18S* was used as an endogenous control in all assays.

Fig. S4. Sulforaphane treatment of prostate cancer cells depletes AR from androgen response elements (AREs) and lowers ERG protein. LNCaP (*A*) or VCaP (*B* and *C*) cancer cells were treated with vehicle or 20 μM sulforaphane for 24 h. ChIP analysis was used to determine AR occupancy at the indicated gene AREs. Enrichment was calculated compared with the respective input control. (*D*) Western blot for AR and ERG protein expression from VCaP whole cell lysates treated in parallel with samples from *B* and *C*. AR, ERG, and actin levels were quantified by Western blot.

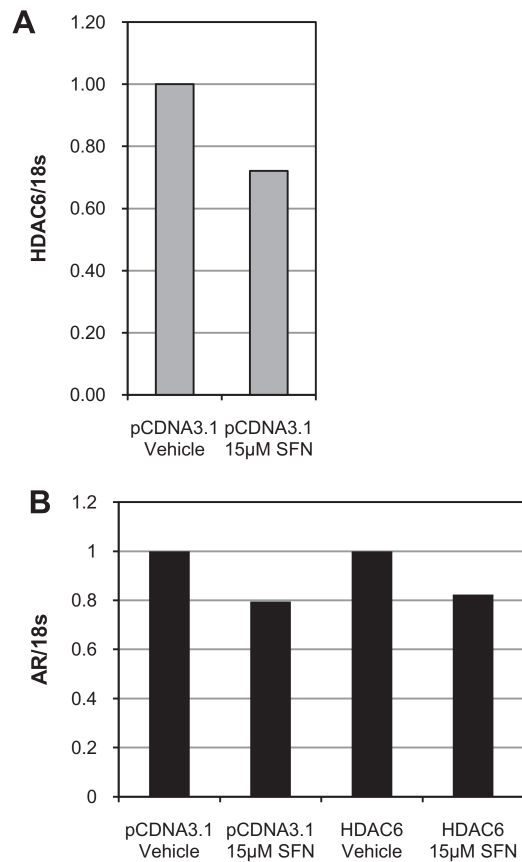

Fig. S5. Sulforaphane treatment of prostate cancer cells lowers *HDAC6* transcript levels whereas HDAC6 overexpression does not increase *AR* transcript levels. LNCaP cells were transfected with pCDNA3.1 or FLAG-HDAC6. Cells were then treated with either vehicle or 15 μM sulforaphane. (*A*) Real-time PCR of *HDAC6* expression. (*B*) Real-time PCR of *AR* expression. The vehicle-treated sample was set to 1. *18S* was used as an endogenous control in all assays.

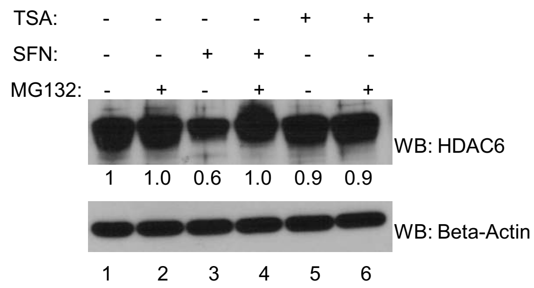

Fig. S6. Proteasome inhibitor treatment of prostate cancer cells rescues HDAC6 protein from sulforaphane treatment. LNCaP cancer cells were treated for 16 h with vehicle without (lane 1) or with (lane 2) 20 μM MG132, 20 μM sulforaphane without (lane 3) or with (lane 4) 20 μM MG132, or 300 nM TSA without (lane 5) or with (lane 6) 20 μM MG132. HDAC6 and actin levels were quantified by Western blot.